Supplementary Information

Properties of the ternary complex formed by yeast eIF4E, p20 and mRNA

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Supp. Fig.1:

S1a) Amino acid sequence of yeast p20 (P12962) showing the canonical eIF4E-binding site (aa 4-10; marked in green) and arginine (aa55-57) or histidine residues (aa60-62) that were mutated to leucine in this work (marked in cyan). The N-terminal 18aa p20 peptide used in this work is underlined.

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  1 MIKYTIDELF QLKPSLTLEV NFDAVEFRAI IEKVKQLQHL KEEEFNSHHV GHFGRRRSSH
 61 HHGRPKIKHN KPKVTTDSDG WCTFEAKKKG SGEDDEEETE TTPTSTVPVA TIAQETLKVK
121 PNNKNISSNR PADTRDIVAD KPILGFNAFA ALESEDEDDE A*
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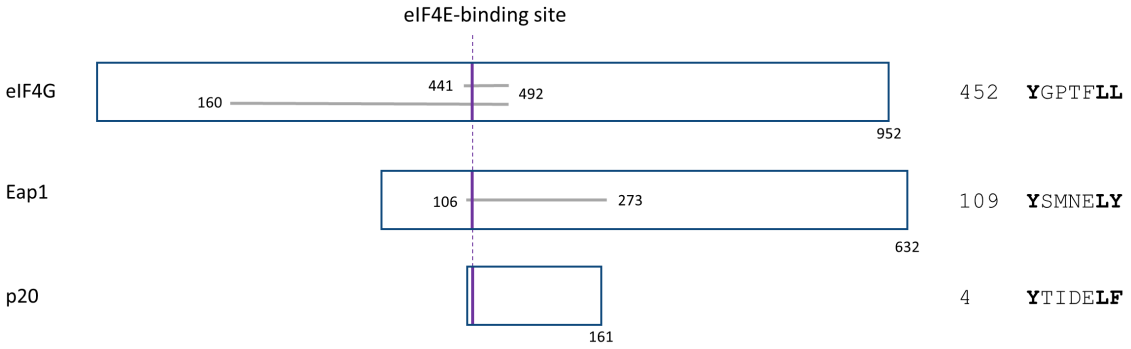
S1b) Schematic depiction of yeast eIF4G, Eap1 and p20 proteins. The canonical YxxxxLL/Y/F eIF4E-binding motif found in each protein is given on the right and its position is indicated by a vertical purple bar. The amino acids corresponding to the eIF4G and Eap1 peptides used in this study are shown as grey horizontal lines.



S1c) Sequence comparison of yeast eIF4E native sequence (top line) vs. codon optimized seqeunce (DAPCEL; bottom line)

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YEAST_eIF4E       ---ATGTCCGTTGAAGAAGTTAGCAAGAAGTTTGAAGAAAACGTTTCAGTCGATGATACC
eIF4E_DAPCEL      ATGGGCAGCGTTGAAGAAGTCTCCAAAAAATTTGAAGAAAACGTTAGCGTAGATGATACT
                           ************   *** ** ***************   ** ********

YEAST_eIF4E       ACAGCTACTCCAAAGACTGTTTTAAGTGACAGTGCTCACTTCGATGTCAAGCACCCATTG
eIF4E_DAPCEL      ACCGCGACCCCGAAAACCGTTTTAAGCGACAGCGCGCACTTCGATGTAAAACACCCGTTA
                  ** ** ** ** ** ** ******** ***** ** *********** ** ***** **

YEAST_eIF4E       AACACCAAATGGACTTTATGGTACACAAAGCCAGCCGTCGATAAATCTGAGTCGTGGTCT
eIF4E_DAPCEL      AATACTAAATGGACCTTATGGTACACCAAACCGGCTGTAGATAAAAGCGAGTCGTGGAGC
                  ** ** ******** *********** ** ** ** ** ******   *********

YEAST_eIF4E       GATCTATTACGTCCCGTCACTTCATTCCAAACTGTTGAAGAATTTTGGGCTATCATTCAA
eIF4E_DAPCEL      GATTTGTTAAGACCCGTAACCAGCTTCCAGACCGTTGAAGAATTTTGGGCGATCATTCAG
                  *** * *** * ***** **    ***** ** ***************** ********

YEAST_eIF4E       AATATTCCTGAGCCACACGAACTACCATTGAAATCAGATTACCACGTCTTCCGTAATGAC
eIF4E_DAPCEL      AATATTCCGGAGCCGCACGAATTGCCGTTAAAAAGCGATTACCACGTATTCAGAAATGAC
                  ******** ***** ****** * ** ** ***   *********** *** * ******

YEAST_eIF4E       GTTAGACCTGAATGGGAAGATGAAGCCAATGCTAAAGGTGGTAAATGGTCTTTCCAACTT
eIF4E_DAPCEL      GTTCGTCCTGAATGGGAAGATGAAGCTAATGCGAAAGGTGGTAAATGGAGCTTCCAGTTG
                  *** * ******************** ***** **************   *****  *

YEAST_eIF4E       AGAGGAAAAGGTGCTGATATTGATGAATTATGGCTAAGAACTTTACTAGCAGTTATTGGT
eIF4E_DAPCEL      CGTGGGAAAGGTGCGGATATTGATGAATTATGGTTGCGTACCTTATTGGCTGTTATTGGT
                   * ** ******** ****************** *  * ** *** * ** *********
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YEAST_eIF4E       GAAACAATTGATGAAGACGACTCCCAAATTAACGGTGTCGTTTTAAGCATTAGAAAAGGT
eIF4E_DAPCEL      GAAACCATTGATGAAGACGACAGCCAGATTAACGGTGTAGTTTTATCCATTCGTAAAGGT
                  ***** ***************  *** *********** ******  **** * ******

YEAST_eIF4E       GGTAACAAGTTTGCCTTATGGACTAAATCTGAAGACAAAGAACCACTATTGAGAATTGGT
eIF4E_DAPCEL      GGTAATAAATTTGCTTTATGGACCAAAAGCGAAGACAAAGAACCGTTGTTACGTATTGGT
                  ***** ** ***** ******** ***   **************  * **  * ******

YEAST_eIF4E       GGTAAATTCAAGCAAGTTTTAAAATTAACCGATGACGGGCATTTGGAATTCTTTCCACAT
eIF4E_DAPCEL      GGTAAATTCAAACAGGTTTTAAAATTAACTGATGACGGGCATTTAGAATTTTTTCCGCAT
                  *********** ** ************** ************** ***** ***** ***

YEAST_eIF4E       TCCAGTGCCAATGGTAGACACCCTCAACCATCAATCACCTTGTAA
eIF4E_DAPCEL      AGCAGCGCTAATGGTCGTCACCCGCAGCCGAGCATCACTTTATAA
                    *** ** ****** * ***** ** **    ***** ** ***
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S1d) Sequence comparison of yeast p20 native sequence (top line) vs. codon optimized sequence (DAPCEL; bottom line)

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YEAST_p20         ATGATCAAGTATACTATCGATGAGCTTTTTCAACTGAAGCCAAGTTTAACTTTGGAAGTT
p20_DAPCEL        ATGATCAAATATACCATCGATGAGTTGTTTCAGCTCAAACCGAGCTTAACTTTAGAAGTT
                  ******** ***** ********* * ***** ** ** ** ** ******** ******

YEAST_p20         AATTTCGATGCGGTGGAATTTAGAGCCATCATTGAAAAAGTTAAGCAATTGCAACACTTG
p20_DAPCEL        AATTTCGATGCTGTAGAATTTCGTGCTATCATCGAAAAAGTTAAACAGTTACAGCACTTA
                  *********** ** ****** * ** ***** *********** ** ** ** *****

YEAST_p20         AAAGAGGAAGAGTTTAACAGTCATCATGTTGGTCATTTCGGTCGTAGAAGATCTTCCCAC
p20_DAPCEL        AAAGAGGAAGAGTTTAACAGCCATCATGTTGGTCATTTCGGTAGACGTCGTAGCTCCCAC
                  ******************** ********************* *  *  *    ******

YEAST_p20         CATCATGGTAGACCAAAGATTAAGCACAACAAGCCTAAGGTTACAACCGATTCAGATGGT
p20_DAPCEL        CATCATGGTCGTCCGAAAATTAAACACAATAAACCGAAAGTTACCACTGATAGCGATGGT
                  ********* * ** ** ***** ***** ** ** ** ***** ** ***   ******


YEAST_p20         TGGTGCACATTTGAAGCCAAGAAGAAGGGTAGTGGAGAAGATGATGAAGAAGAAACAGAA
p20_DAPCEL        TGGTGCACCTTTGAAGCTAAAAAAAAAGGTAGCGGGGAAGATGATGAAGAAGAAACCGAA
                  ******** ******** ** ** ** ***** ** ******************** ***

YEAST_p20         ACCACACCAACTTCTACTGTGCCAGTTGCTACCATTGCCCAAGAAACTTTAAAAGTCAAG
p20_DAPCEL        ACTACCCCGACCAGCACCGTACCGGTTGCGACTATTGCTCAGGAAACCTTAAAAGTAAAA
                  ** ** ** **    ** ** ** ***** ** ***** ** ***** ******** **

YEAST_p20         CCAAATAACAAAAATATTTCTTCCAACAGACCTGCTGATACCAGAGATATTGTTGCGGAC
p20_DAPCEL        CCGAATAACAAAAATATCTCTTCCAACCGCCCCGCGGATACTCGTGATATTGTTGCTGAC
                  ** ************** ********* * ** ** *****  * ********** ***

YEAST_p20         AAGCCAATTCTTGGTTTCAACGCATTTGCTGCTTTGGAAAGTGAAGACGAAGACGACGAA
p20_DAPCEL        AAACCGATTTTGGGTTTCAATGCTTTTGCGGCGTTAGAAAGCGAAGACGAAGACGACGAA
                  ** ** *** * ******** ** ***** ** ** ***** ******************

YEAST_p20         GCATAA
p20_DAPCEL        GCTTGA
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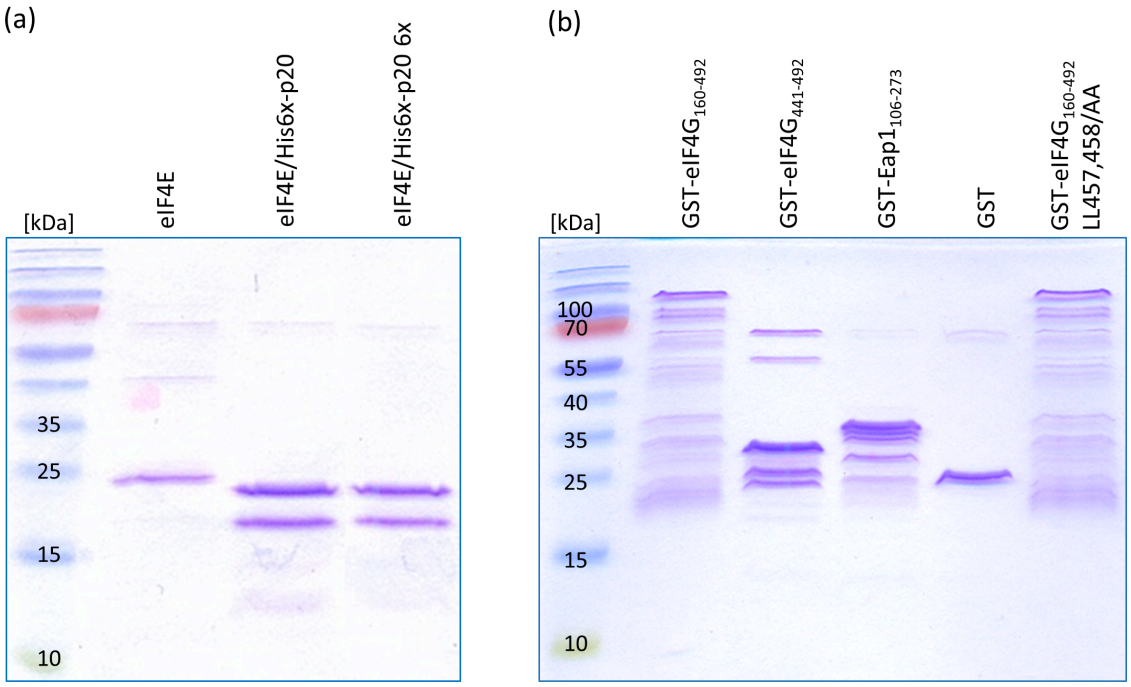
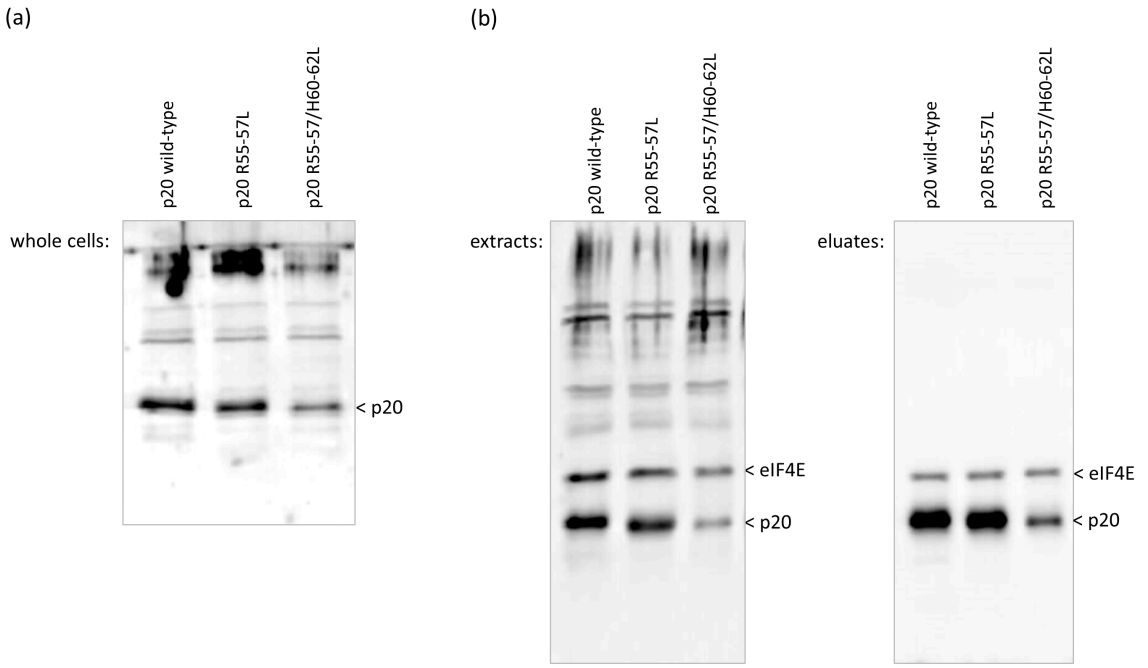
S1e) Nucleotide sequence of RNAs used in this work:

3'FAM labeled 40 nt SSA1 RNA (Microsynth AG), used for MicroScale Thermophoresis™ or Electrophoretic Mobility Shift Assay measurements	GUAUUACAAGAAACAAAAAUUCAAGUAAAUAACAG AUAAU
In vitro transcribed, m^7GTP- or m^7GpppG-capped 64 nt SSA1 5'UTR RNA, used for MicroScale Thermophoresis™ or Electrophoretic Mobility Shift Assay measurements	GGGAACAAAAGCUGGUAAUCAAGAAUUACAAGAAA CAAAAAUUCAAGUAAAUAACAGAUAAUAC
"Average" 5'UTR fused to Renilla Luciferase ORF (AUG marked in bold), used for *in vitro* translation assays	GGGAACAAAAGCUGGAGCUCGCCCGGGCUGUUCUA GCCACC**AUG**G

PMA1 5'UTR fused to Renilla Luciferase ORF (AUG marked in bold), used for *in vitro* translation assays	GGGAACAAAAGCUGGAGCUCGCCCGGGACACCAAU AGUGAAAAUCUUUUUUUCUUCUAUAUCUACAAAAA CUUUUUUUUUCUACAAAAACUUUUUUUUUCUAUCA ACCUCGUUGAUAAAUUUUUUCUUUAACAAUCGUUA AUAAUUAAUUAAUUGGAAAAUAACCAUUUUUUCUC UCUUUUAUACACACAUUCAAAAGAAAGAAAAAAAA UAUACCCCAGCUAGUUAAAGAAAAUCAUUGAAAAG AAUAAGAAGAUAAGAAAGAUUUAAUUAUCAAACAA UAUCAAUCC**AUG**G
GRE1 5'UTR fused to Renilla Luciferase ORF (AUG marked in bold), used for *in vitro* translation assays	GGGAACAAAAGCUGGAGCUCGCCCGGGGAGCCAAA CACUACCGCAUAAAAGCUAAGUACGAAUAACAAUU AAGAACC**AUG**G
SSA1 5'UTR fused to Renilla Luciferase ORF (AUG marked in bold), used for *in vitro* translation assays	GGGAACAAAAGCUGGUAAUCAAGAAUUACAAGAAA CAAAAAUUCAAGUAAAUAACAGAUAAUACC**AUG**G



Supp. Fig.2: Coomassie stained SDS-PAGE (20%)
(S2a) 0.75 µg purified His6x-eIF4E alone (lane 1), 2.0 µg co-expressed and purified eIF4E/His6x-p20 complex (lane 2), and 2.0 µg co-expressed and purified eIF4E/His6x-p20 6x mutant complex (lane 3)
(S2b) 4 µg GST-eIF4G$_{160-492}$ (lane 1), 4 µg GST-eIF4G$_{441-492}$ (lane 2), 4 µg GST-Eap1$_{106-273}$ (lane 3), 4 µg GST (lane 4), and 4 µg GST-eIF4G$_{160-492}$ LL457,458/AA mutant (lane 5).



Supp. Fig. 3: Full-length Western blots (sections of which are presented in Figure 2d). Blots were stained with polyclonal antibodies against eIF4E or p20 as described in Methods.
S3a) Western blot of ½ OD_{600} whole yeast cells carrying p20 knockout (RH2585 Δp20) and expressing p20 wild type, R55-57L (3x) or R55-57L, H60-62L (6x) mutants.
S3b) Western blots of input extracts (10 µg loaded on blot) used for m^7GDP-Sepharose pulldown (left panel) and eluates (right panel) for p20 wild type, R55-57L (3x) and R55-57L, H60-62L (6x) mutants.